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Spatial variability of nitrous oxide in the Minho and Lima estuaries (Portugal)

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Summary: Nitrous oxide (N_2O) is a potent long-lived greenhouse gas and estuaries represent potentially important sources of this biogas to the atmosphere. In this work, we analyse the first N_2O data obtained in the Minho and Lima estuaries, and the processes and environmental factors that may regulate its production in these systems. In September 2006, N_2O attained values of up to 20.0 nmol L^{-1} in the upper reaches of the Lima estuary and the river was, apparently, the main source of biogas to the system. In Minho N_2O reached a maximum of 14.4 nmol L^{-1} and nitrification appeared to contribute to the enhancement of N_2O. In the upper estuary, the relatively high concentrations of nitrification substrate NH_4^+, the positive correlations found between N_2O level above atmospheric equilibrium (ΔN_2O) and apparent oxygen utilization and NO_2^-, and the negative correlations between ΔN_2O and NH_4^+ and pH can be interpreted as in situ N_2O production through pelagic nitrification. Principal component analysis gave evidence of considerable differences between upper estuaries, particularly in terms of higher N_2O in Lima and NH_4^+ in Minho. Surface waters of both estuaries were always N_2O-supersaturated (101-227%) and estimated N_2O emissions from Minho and Lima were 0.28 Mg N_2O-N yr^{-1} and 0.96 Mg N_2O-N yr^{-1}, respectively, which represent a reduced fraction of N_2O global emission from European estuaries.

Keywords: N_2O; greenhouse gas; fluxes; emission; Portuguese estuaries.

Variabilidad espacial del óxido nitroso en los estuarios del Miño y Lima (Portugal)

Resumen: El óxido nitroso es un poderoso gas de efecto invernadero y los estuarios representan potenciales fuentes de este biogás a la atmósfera. En este trabajo se analizan los primeros datos de N_2O obtenidos en los estuarios de Miño y Lima, así como los procesos y factores ambientales que pueden regular su producción en estos sistemas. En septiembre de 2006, N_2O alcanzó en el tramo superior del Lima valores de hasta 20,0 nmol L^{-1} y el río Lima fue, aparentemente, la principal fuente de biogás para el sistema. En el Miño N_2O alcanzó una concentración máxima de 14,4 nmol L^{-1} y la nitrificación parece contribuir al aumento de N_2O. En el tramo superior del Miño las concentraciones relativamente altas del substrato de nitrificación NH_4^+ y las correlaciones positivas encontradas entre el exceso de N_2O (ΔN_2O) y el consumo aparente de oxígeno (AOU) y NO_2^-, y las correlaciones negativas entre ΔN_2O, NH_4^+ y pH pueden interpretarse como una producción in situ de N_2O debido a la nitrificación pelágica. El Análisis de Componentes Principales evidenció diferencias considerables entre la cabecera de los estuarios particularmente en términos de mayor concentración de N_2O en el Lima y NH_4^+ en el Miño. Las aguas superficiales del Miño y del Lima presentaban sobresaturación de N_2O (101-227%) y las emisiones estimadas de N_2O fueron de 0,28 Mg de N_2O-N $año^{-1}$ y 0,96 Mg de N_2O-N $año^{-1}$, respectivamente, que representan una fracción reducida de las emisiones totales de N_2O procedentes de los estuarios europeos.

Palabras clave: N_2O; gas de efecto invernadero; flujos; emisión; estuarios portugueses.

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INTRODUCTION

In the last few decades, the study of N_2O has acquired greater importance due to its contribution to global climate change. N_2O is an important long-lived greenhouse gas in terms of radiative forcing (0.17±0.03 W m^{-2}) (Myhre et al. 2013) and represents the major anthropogenic contributor to stratospheric ozone destruc-

tion. It has a long atmospheric lifetime of 131±10 years (Prather et al. 2012) and its global warming potential is 310 times greater than that of carbon dioxide, in a time horizon of 100 years. In 2011 atmospheric N_2O levels (324.2±0.1 ppb) exceeded the pre-industrial levels (270±7 ppb) by about 20% (Myhre et al. 2013), largely due to increased agricultural activity and industry.

Estuaries have been considered significant N_2O contributors to the atmosphere as a consequence of their high productivity and anthropogenic nitrogen loadings. N_2O is mainly formed during the first step of nitrification, the aerobic oxidation of ammonium (NH_4^+) to nitrite (NO_2^-), mediated by ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA), and microbiological denitrification, the biological reduction of nitrate (NO_3^-) to N_2O, and, in turn, nitrogen gas (N_2). Nitrification and denitrification often occur simultaneously in aquatic ecosystems and their relative contribution to total N_2O production is difficult to disentangle. As nitrification is an aerobic process in well-oxygenated estuarine systems, the water column mostly contributes N_2O through nitrification production (de Wilde and de Bie 2000, Barnes and Upstill-Goddard 2011). Denitrification is usually limited to zones under hypoxic conditions (DO <2 ml L^{-1}), although some denitrification may occur even in relatively oxygenated waters (de Bie et al. 2002).

Both nitrification and denitrification are sensitive to the ongoing environmental changes and any natural or anthropogenic-induced shifts in the N availability have the potential to alter nitrogen cycling in coastal environments and affect N_2O formation and release to the atmosphere (Bange et al. 2010). The extent of the denitrification is also strongly controlled by temperature, nitrate concentrations and the availability of organic carbon (e.g. Dong and Nedwell 2006). In addition to the supply of oxygen and ammonia, which are the main controls on nitrification, other environmental variables may affect this biological process: temperature (Dai et al. 2008), salinity (Bollmann and Laanbroek 2002) and pH (Strauss et al. 2002).

Estimates of N_2O release from estuaries to the global inventory reveal wide uncertainties due to the large variability in N_2O data (Bange et al. 2010, Barnes and Upstill-Goddard 2011). However, considerable efforts have been made in the last few decades to better understand the nitrogen cycle, the dynamics of N_2O production and the quantification of the respective emission from European estuaries. More recently Murray et al. (2015) reviewed N_2O global fluxes from estuarine environments and reported a variation of 0.17-0.95 Tg N_2O-N yr^{-1}.

Studies on the N_2O dynamics and fluxes have been carried out in the Portuguese Tagus, Sado and Douro estuaries (e.g. Gonçalves et al. 2010, 2015, Teixeira et al. 2013). However, no data on N_2O levels and fluxes are available for the Minho and Lima estuaries. In this work, we (1) report spatial variability of N_2O concentration in these systems, (2) assess the contribution of different N_2O sources, (3) evaluate the role of environmental properties on the increment of N_2O fluxes, and (4) estimate N_2O emission in a perspective of global N_2O estuarine emissions.

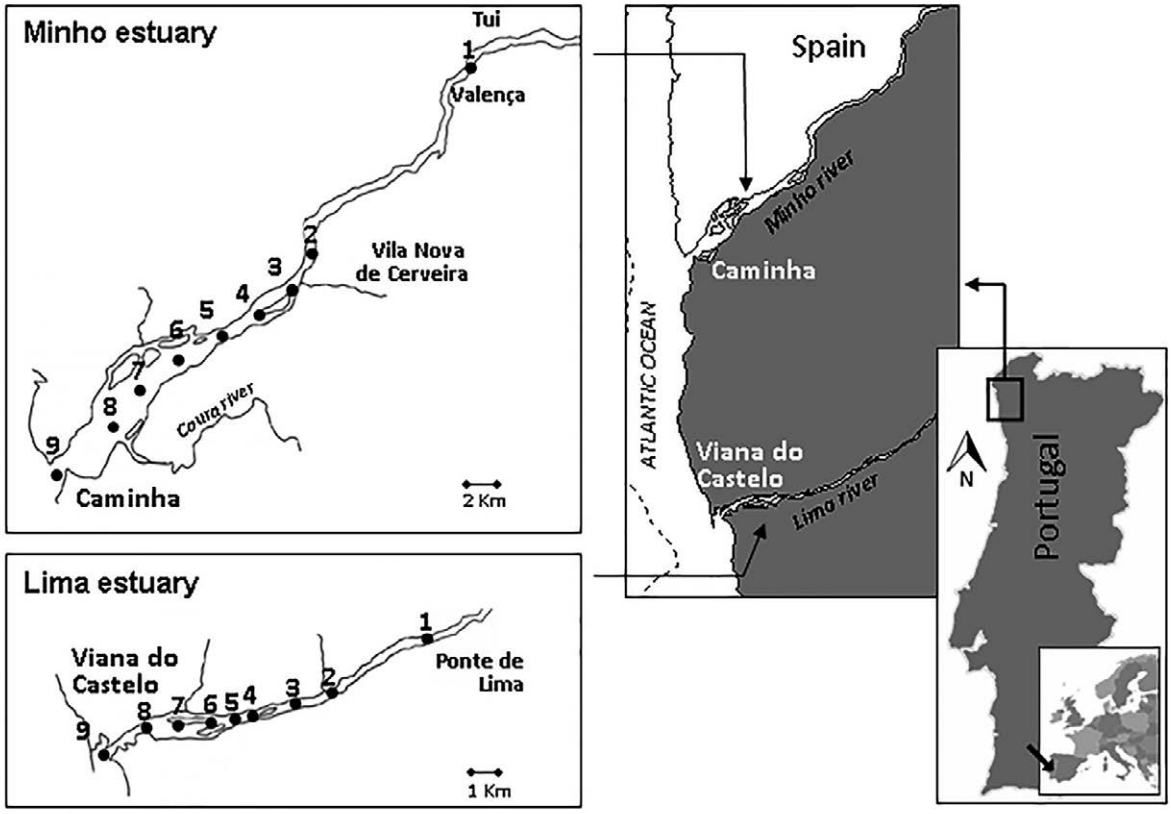

Fig. 1. – Map of the Minho and Lima estuaries. Dots and numbers represent sampling sites.

Table. 1 – Main physical and hydrological characteristics of the Minho and Lima estuaries (Ferreira et al. 2005).

Estuary	Area (km^2)	Mean depth (m)	Mean water volume ($\times 10^6$ m^3)	Mean residence time (days)	Mean tidal range (m)	Mean annual freshwater input (m^3 s^{-1})	Watershed population density (hab km^{-2})	N load (t yr^{-1})
Minho	23	2.6	70	1.5	3	300	112	20051
Lima	5	4.0	19	1	2	54	87	1078

MATERIALS AND METHODS

Area description

The Minho and Lima estuaries, both situated in the northern part of Portugal (Fig. 1), differ essentially in terms of river discharge, anthropogenic pressures and morphology (Table 1).

Minho estuary

The Minho estuary is situated in the border region between Portugal (Minho region) and Spain (Galicia region). The estuary has an area of approximately 23 km^2, a mean depth of 2.6 m, and a maximum width of about 2 km at the confluence of the Coura River, at Caminha. The estuary is mesotidal, with a mean tidal range of 3 m, and its dynamic tidal effects extends 35 km upstream (Bettencourt et al. 2003).

The annual mean discharge of the Minho River is 300 m^3 s^{-1} (Table 1), ranging between approximately 100 m^3 s^{-1} in August and 800 m^3 s^{-1} in February, and the water residence time is 1.5 days (Sousa et al. 2013). The estuary has high ecological value, mainly due to the large diversity of habitats and biodiversity and great socio-economic importance from tourism, fishing and agriculture. However, increasing pressure has been detected over recent years, involving in particular the pollution of surface water from both specific and diffuse sources, morphological alterations, changes of land use in the drainage basin and other impacts from human activity, such as aquaculture, textile, rubber and plastic processing industries (Ferreira et al. 2005). The Minho and Coura rivers are the main nitrogen sources to the estuary, contributing 13000 t N yr^{-1} and 7000 t N yr^{-1}, respectively, and effluents from domestic origin contribute 51 t N yr^{-1} (Ferreira et al. 2005) (Table 1).

Lima estuary

The Lima estuary, located south of Minho, has an area of approximately 5 km^2 and a mean depth of 4 m (Table 1). It has a semidiurnal mesotidal regime and the tidal effect extends 20 km upstream (Ramos et al. 2006). The upper estuary is constituted by a narrow channel with some intertidal areas and undisturbed banks. The middle estuary is a shallow salt marsh zone, and the lower estuary consists of a wider and shallow basin that communicates with the sea via a deep, narrow channel (with a typical depth of about 10 m). The estuary mouth is artificially obstructed by a 2-km-long jetty. The Lima River has an annual freshwater discharge of 54 m^3 s^{-1}, and the mean water residence time in the estuary is one day (Table 1).

The lower estuary is a highly urbanized zone and has been subjected to several anthropogenic impacts resulting from harbour activities (Viana do Castelo). However, nutrient loadings also originate from diffuse sources, largely agriculture (0.51 t N yr^{-1}), although the main N source to the estuary is the Lima River (1077 t N yr^{-1}) (Ferreira et al. 2005). This N load is, however, approximately 19 times lower than the load to the Minho estuary (Table 1).

Sampling

Water sampling was undertaken in September 2006 during ebb tide (at spring tide), at nine stations located along a main transect of both estuaries, from the upstream limit of tidal influence to the estuary mouth, covering a full range of salinity of 0-30 corresponding to a distance of 26.5 km in Minho and 15 km in Lima estuary (Fig. 1).

Surface water (0.2 m depth) was collected using 2-L Niskin bottles (General Oceanics) for analysis of salinity (S), temperature (T), pH, dissolved inorganic nitrogen (nitrate NO_3^-, nitrite NO_2^- and ammonium NH_4^+), dissolved oxygen (DO) and nitrous oxide (N_2O). The hydrological characteristics of the Minho and Lima estuaries and the meteorological conditions observed during the sampling period are presented in Table 2.

Analytical procedure

Water temperature (T) was measured in situ with a Seabird SBE19/CTD probe with an accuracy of 0.01°C.

Salinity (S) measurements were carried out using a temperature-controlled Guideline Salinometer (Portasal 8410A), and accuracy was 0.03 salinity. Equipment was calibrated with a certified IAPSO Standard Seawater reference.

Meteorological parameters (air temperature, pressure, and wind speed and direction) were determined using a portable meteorological station (Campbell Scientific CR510). Measurements represent the average of physical parameters taken using a sampling time of 5 seconds and a storage time of 1 minute. Wind speed 10-minute average was determined for each sampling

Table 2. – Hydrological characteristics of the Minho and Lima estuaries and meteorological conditions observed in September 2006. Q, daily mean flow of the Minho and Lima rivers measured at Foz Mouro and Ponte da Barca, respectively (SNIRH 2013); u_{10}, daily wind speed normalized to 10 m height.

Estuary	Sampling date	Tidal amplitude (m)	Q daily mean (m^3 s^{-1})	u_{10} (m s^{-1})
Minho	12 Sep	0.6-3.3	90.7	2.5-6.2
Lima	13 Sep	0.9-3.1	8.1	1.3-4.1

station and converted to wind speed values at 10 m height (u_{10}) using a logarithmic correction (Hartman and Hammond 1985).

The Minho and Lima River discharges were calculated as an average of the flow 10 days before sampling, at the hydrometric stations of Foz de Mouro and Ponte da Barca, respectively (SNIRH 2013).

Dissolved oxygen (DO, μmol L^{-1}) was measured using whole-bottle Winkler's titration method (Aminot and Chaussepied 1983). A Methrom titrator was used to dispense small amounts of thiosulphate, and starch endpoint was detected visually. Precision of the method was in the range of 0.08% to 0.25%. DO saturation, expressed in percentage (%), was determined as the ratio of the oxygen concentration determined and the equilibrium values of DO calculated with the Weiss (1970) equation. Apparent oxygen utilization (AOU, μmol L^{-1}) was calculated as the difference between the saturation oxygen concentration and the dissolved oxygen concentration measured in the sample.

Water samples for determination of dissolved inorganic nitrogen were filtered through acetate cellulose filters (pore size 0.45 μm) and stored at –20ºC until analysis. Analyses were carried out using a Traacs autoanalyser following colorimetric techniques outlined by the manufacturer. Estimated precision was ±0.8% for nitrate and nitrite (NO_3^- and NO_2^-) and ±2.0% for ammonium (NH_4^+), at mid-scale concentrations. Accuracy of nutrient measurements was maintained by using CSK Standards (WAKO, Japan).

pH measurement was carried out immediately after collecting water samples using a Metrohm 704 pH-meter and a combined electrode (Metrohm), standardized against NBS buffers (6.865 and 9.180 pH). Precision of pH measurements was ±0.01.

Water samples for determination of N_2O were collected in triplicate in 20-mL glass headspace vials and poisoned with saturated aqueous mercury chloride ($HgCl_2$) to stop biological activity. The vials were stored upside down, in the dark, at 4°C in the refrigerator until analysis, performed within 10 days. Dissolved N_2O was determined by a headspace equilibration technique coupled with gas chromatographic analysis (GC-3800, Varian). Briefly, 20 mL of sample was equilibrated with 5 mL of highly purified helium (purity =99.9999%) in a headspace CombiPAL autosampler. Gas chromatographic separation was carried out using a stainless steel column packed with 80/100 (mesh) Porapak. Oven and detector temperature was set at 50°C and 320°C, respectively, and high purity nitrogen (99.9999%) was used as the carrier gas (flow rate 30 mL min^{-1}). To remove water vapour and carbon dioxide, absorbent columns packed respectively with $Mg(ClO_4)_2$ and Carbosorb were located in the carrier gas line between the sample loop and the separation column. N_2O peak was detected with a ^{63}Ni electron capture detector (ECD). Calibration of ECD response was performed using standard gas mixtures with 400, 780 and 1980 ppb N_2O in synthetic air (Air Liquide), and method precision was 2.6% (30 replicate measurements using samples containing 10 nmol L^{-1} of N_2O). In situ concentration of N_2O (C, nmol L^{-1}) was calculated from the concentrations measured in the headspace according to the solubility equation of Weiss and Price (1980):

$$C = \beta\,(TS)\,x'P$$

where x' is the measured N_2O dry mole fraction, P is the atmospheric pressure, and β is the solubility coefficient, which is a function of the water temperature (T) and salinity (S). N_2O equilibrium concentrations were calculated assuming an atmospheric N_2O mixing ratio of 320.1 ppb (WMO 2006).

N_2O saturation, expressed in percentage (%), was determined as the ratio between the measured dissolved N_2O concentration and the equilibrium concentration. The N_2O water-air flux (F_{N_2O}) was estimated according to the following equation:

$$F_{N_2O} = k_{N_2O}\,\Delta N_2O$$

where ΔN_2O, the excess of N_2O, is the difference between the measured concentration and the equilibrium concentration with the atmosphere in the estuarine water at the local temperature and salinity; and k_{N_2O} (cm h^{-1}) is the N_2O transfer velocity, which is expressed as a function of the wind speed and the Schmidt number (Sc). Since no direct measurements of k_{N_2O} were made in the Minho and Lima estuaries, both the k-wind relationships of Carini et al. (1996) (hereinafter referred to as C96) and Raymond and Cole (2001) (hereinafter referred to as RC01) were, respectively, used to compute k:

$$k_{C96} = 0.045 + 2.0277u_{10}$$

$$k_{RC01} = 1.91\ e^{0.35u_{10}}$$

The gas transfer velocities and air-sea fluxes were estimated using in situ wind speeds normalized to 10 m height (u_{10}). The k coefficients were corrected for in situ temperature using the following relationship:

$$k_{N_2O}/k_{600} = (Sc_{N_2O}/600)^{-0.5}$$

where Sc_{N_2O} is the Schmidt number for N_2O calculated according to the equation of Wanninkhof (1992):

$$Sc = 2301.1 - 151.1\,T + 4.7364\,T^2 + 0.057431\,T^3$$

where T is the temperature (ºC).

Statistical analysis

An unpaired t-test was used to identify statistical differences in levels of N_2O and other environmental variables between and along estuaries.

Pearson's correlation analyses were performed to evaluate the existence of relationships between ΔN_2O and the variables NH_4^+, AOU, NO_2^- and pH assumed to be connected with production pathways of this biogas.

The multivariate techniques principal component analysis (PCA) and cluster analysis were applied to

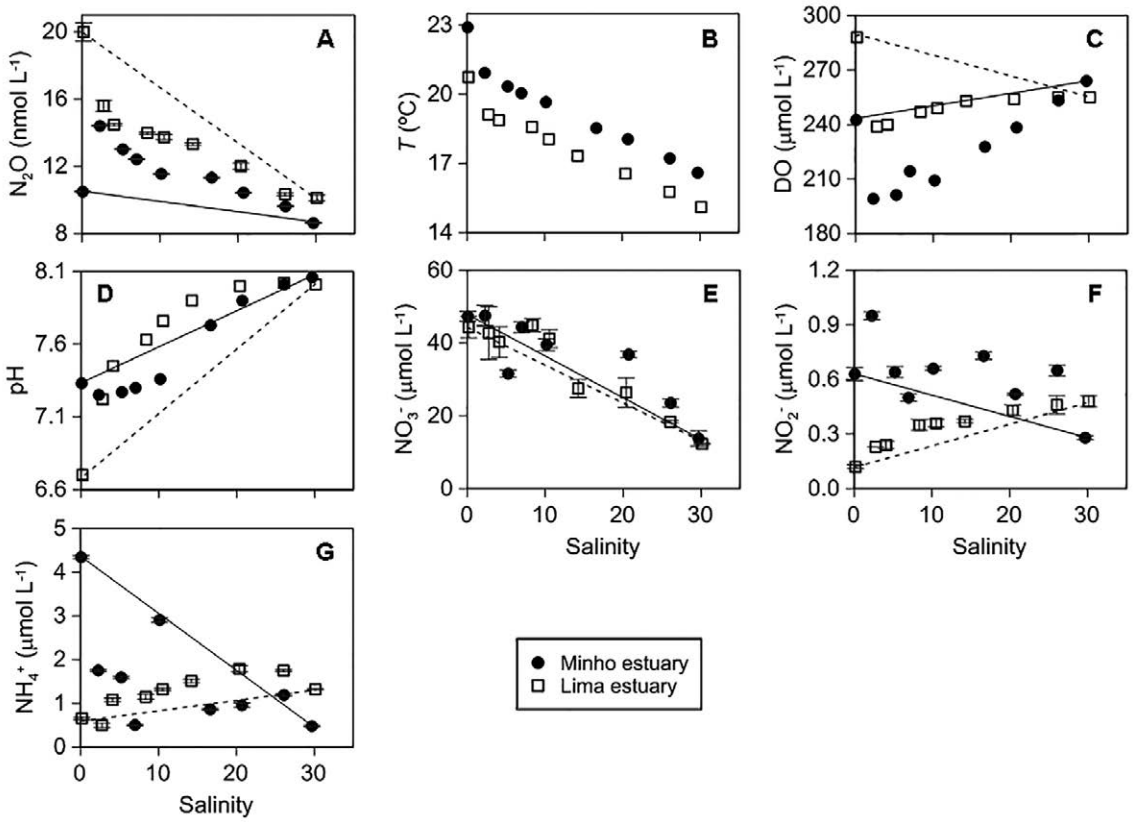

Fig. 2. – N_2O and other environmental parameters against salinity in the Minho (black circles) and Lima (open squares) estuaries (error bars=±1 standard deviation, sd). Conservative lines (solid line, Minho; dashed line, Lima) are shown when appropriate.

environmental data in order to identify and compare inter-relationships between these variables in both estuaries. Data were log(x+1) transformed and handled using correlation-based PCA on the basis of standard Euclidean distance between samples to define their dissimilarity. PRIMER (version 6) was employed for the multivariate analysis.

Figures were created in Golden Software Grapher program (version 9.6.1001).

RESULTS

N_2O levels and fluxes

Concentrations of N_2O and the studied environmental parameters plotted against salinity along both Minho and Lima estuaries are shown in Figure 2.

Distribution of N_2O exhibits a declining tendency towards the mouths of both estuaries. In general, concentrations were higher in Lima than in Minho (Fig. 2A). In Lima values ranged from 10.1 to 20.0 nmol L^{-1} and were below the conservative mixing line. The maximum value was reached in the upper estuary, suggesting that the Lima River was the main source of N_2O to the estuarine system. From salinity 0 to 4 a sharp decrease was observed, suggesting a greater N_2O loss in the zone, presumably through water-air gas exchange. Downstream of salinity 4, N_2O decreased more slightly and the mixing with the N_2O-poorer seawater is well perceptible. In the Minho estuary, concentrations varied from 8.6 to 14.4 nmol L^{-1} and by contrast with those observed in Lima were above the conservative mixing line, pointing to the existence of N_2O sources within the estuary. A major concentration increase from 10.5 to 14.4 nmol L^{-1} was detected between 0 and 2.5 salinity, suggesting the existence of internal N_2O sources in this zone of the estuary, presumably from manufacturing industries located nearby. N_2O saturation values ranged from 101% to 166% along the Minho estuary and from 113% to 227% along the Lima estuary (see Table 4), indicating that both estuaries are potential N_2O sources to the atmosphere.

Surface waters of both estuaries were well oxygenated during our study period. Concentrations of DO were higher than 280 µmol L^{-1} in the upper Lima estuary at salinity 0.2, decreasing to 240 µmol L^{-1} at salinity 2.8 (Fig. 2C). Seawards of this salinity, an increasing tendency was observed and a concentration of 255 µmol L^{-1} was also reached in the vicinity of the estuary mouth (salinity 30.2). In the Minho estuary, a decrease in DO was also detected in the upper estuary and the concentration dropped from 243 µmol L^{-1} at 0.1 salinity to 199 µmol L^{-1} at 2.3 salinity. Afterwards, a sharp increase in DO was measured along the estuary and a maximum value of 264 µmol L^{-1} was reached at the estuary mouth (salinity 29.7). DO-enriched seawater probably accounted for the similar increasing trend seaward in both estuaries. Saturation values were higher than 70% in the Minho estuary and 90% in the Lima estuary.

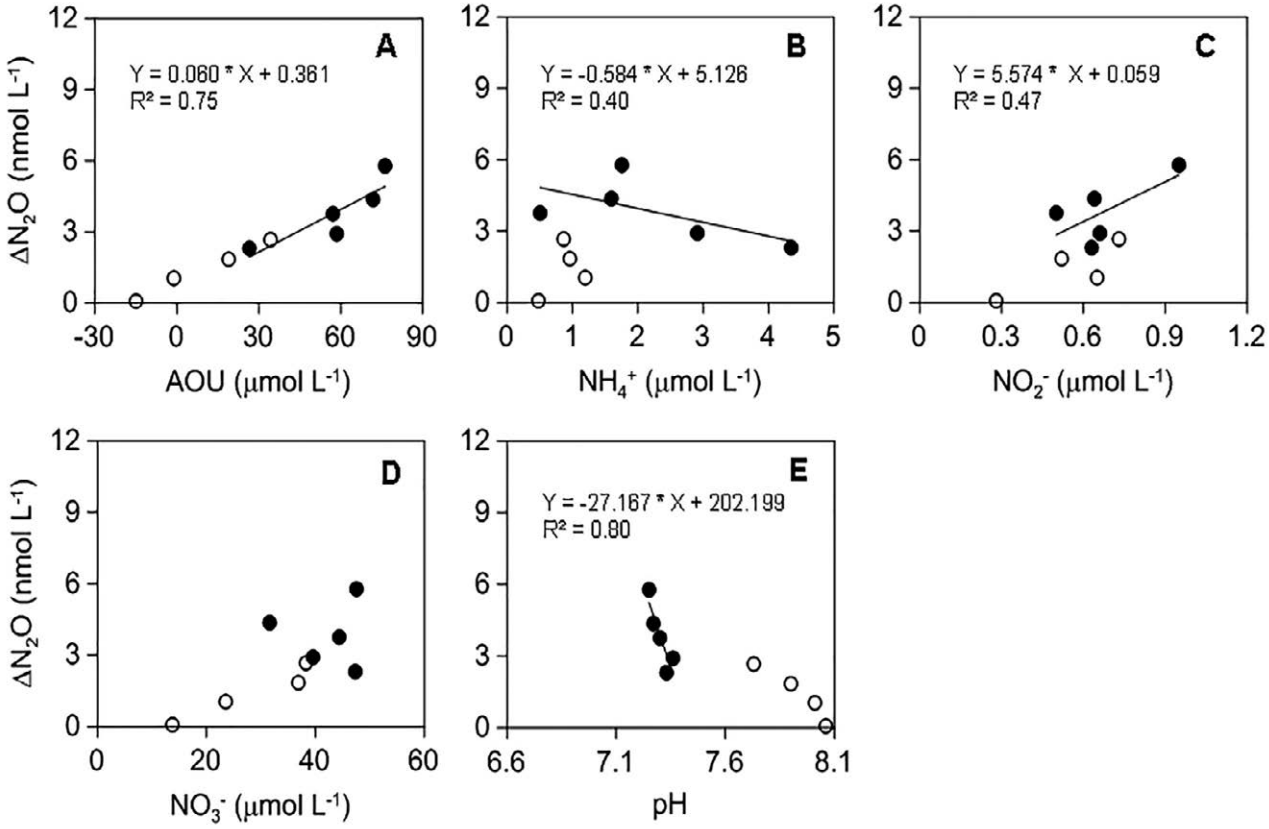

Fig. 3. – Relationships between ΔN_2O and environmental parameters in the Minho estuary. Lower salinity sites (0-10 salinity) are represented by black circles and higher salinity sites (15-30 salinity) by open circles. R^2, correlation coefficient.

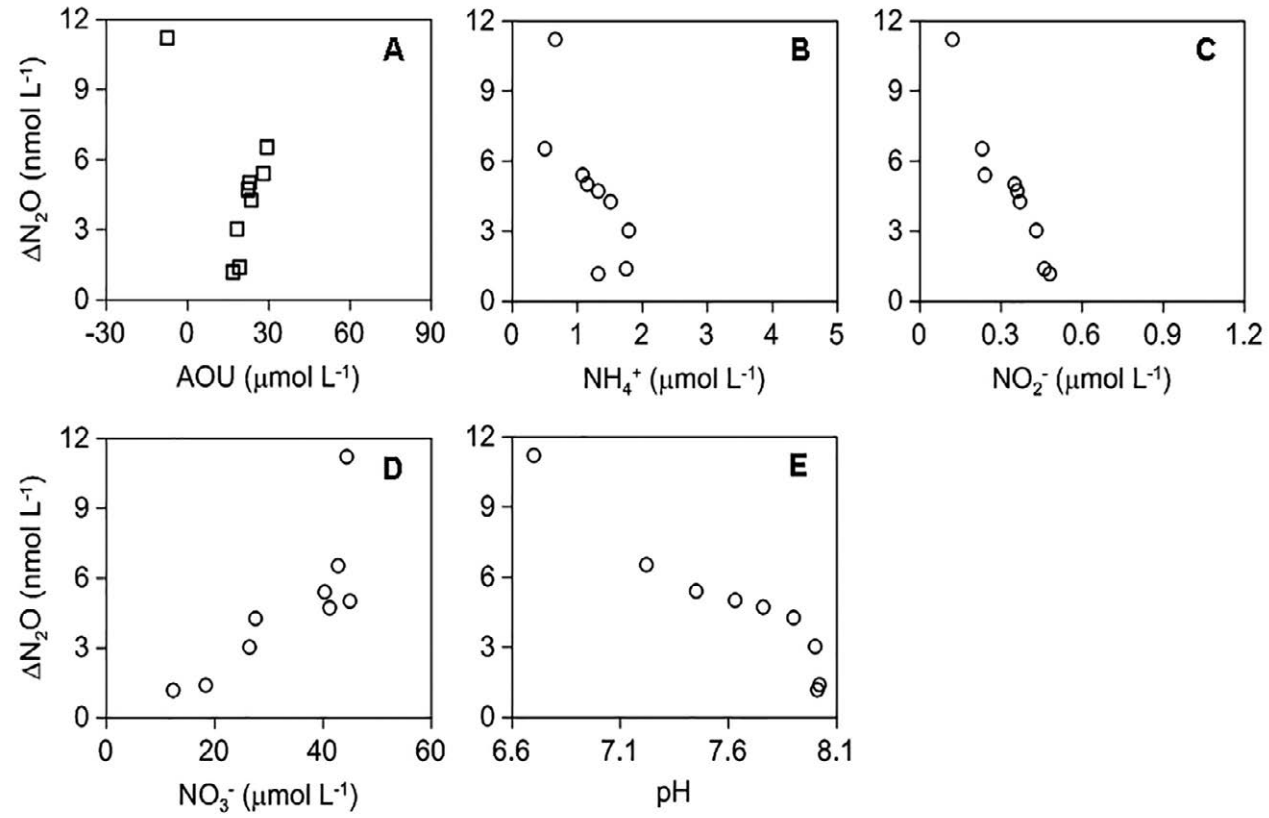

Fig. 4. – Relationships between ΔN_2O and environmental parameters in the Lima estuary.

Table 3. – Results of principal component analysis showing loadings of variables for the first two principal components for the Minho and Lima estuaries.

Environmental variable	PC1	PC2	PC3
Eigenvalues	4.27	2.17	0.74
% Variation	53.4	27.1	9.7
Cum. % Variation	53.4	80.6	90.2

In the Minho estuary pH increased from 7.3 in the more river-influenced zone to 8.1 at the estuary mouth (Fig. 2D). In the Lima estuary pH showed a larger range of values, increasing from 6.7 in the most river-influenced site to 8.0 at the estuary mouth.

NO_3^- was the dominant species of inorganic nitrogen in both estuaries, reaching a similar maximum concentration in the river input (47.6 μmol L^{-1} in Minho and 44.4 μmol L^{-1} in Lima) (Fig. 2E). Values decreased seawards and in general followed the theoretical conservative mixing line. Along the Minho estuary both NO_2^- and NH_4^+ exhibited an irregular behaviour, though the system seemed to function as an NO_2^- source and an NH_4^+ sink (Fig. 2F, G). Between salinity 0 and ~5-7 the decline in NH_4^+ (~4.0 to 0.5 μM L^{-1}) was simultaneous with an increase in NO_2^- (~0.6 to 1.0 μM L^{-1}) and N_2O (~10 to 13-14 nmol L^{-1}), suggesting the occurrence of nitrification.

Figure 3 displays relationships between ΔN_2O and AOU, NH_4^+, NO_2^-, NO_3^- and pH in the Minho estuary. In the mentioned salinity zone (0 and ~5-7) a significant positive correlation was found between ΔN_2O and AOU (R^2=0.75). This indicates the occurrence of nitrification as a source of N_2O and the respective slope provides an estimate of the biological N_2O yield per mole O_2 consumed (Yoshinari 1976). Further, the simultaneous (negative) correlations between ΔN_2O and the primary substrate for nitrification, NH_4^+ (R^2=0.40), and pH (R^2=0.80), and the positive correlation between ΔN_2O and the byproduct of nitrification, NO_2^- (R^2=0.41), are consistent with the predominance of nitrification as a mechanism of N_2O production in the upper part of the Minho estuary. No correlation was found with NO_3^-, whose high concentrations were mostly riverine derived.

In the Lima estuary no relationships were found, suggesting the occurrence of nitrification (Fig. 4).

The application of PCA to the studied environmental variables in the Minho and Lima estuaries allowed us to identify two main composite variables, PC1 and PC2 (eigenvalues >1.0), which explain 82% of the variance (Table 3) and represent a good description of the environmental structure across the estuarine sampled sites.

PC1 explained 53% of variance and had the highest positive loading for NO_3^-, T and N_2O and a negative loading for S and pH (Fig. 5). This component represents the separation of major river-influenced stations from major marine-influenced ones in both estuaries. PC2 explained 27% of the variance and correlated positively with DO and negatively with NO_2^- and NH_4^+. This component appears to represent relevant parameters to N dynamics, particularly in Minho estuary. In fact, projection of stations along PC2 reveals a clear separation of sites from the upper Lima estuary (L1-L5) (Fig. 5), mostly associated with higher values of N_2O, and sites from the upper Minho (M1-M5) more associated with higher NO_2^- and NH_4^+, apparently from river origin, as the N load from the Minho River is considerable at this point (Table 1). It was also observed that the stations from both middle/lower estuaries did not differ in terms of studied environmental variables.

N_2O water-air fluxes are shown in Figure 6. Positive values prevailed at all stations but decreased, in general, between the upper and lower zone of the estuaries. This tendency was more pronounced in the Lima estuary, where higher N_2O fluxes in the river-influenced area were about twice (~12.0 μmol m^{-2} d^{-1}; C96) (Fig. 6B) those observed in the upper part of the Minho estuary (~6.0 μmol m^{-2} d^{-1}; C96) (Fig. 6A). The

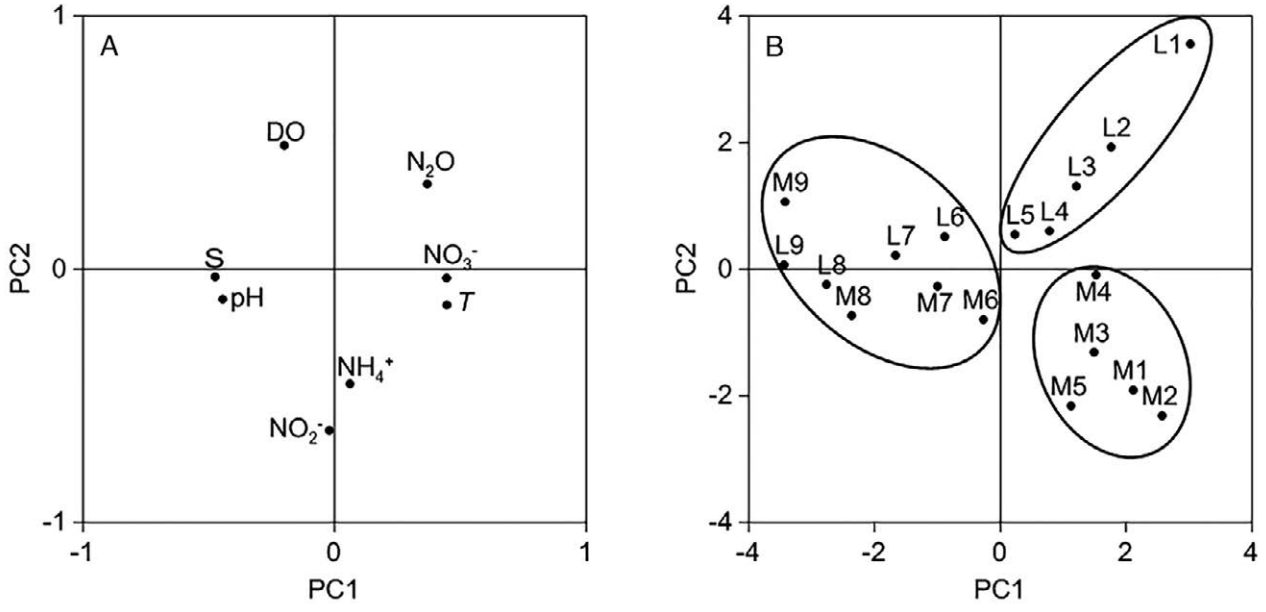

Fig. 5. – PCA ordination of variables loadings (A) and scores of sampling stations (B) in the Minho and Lima estuaries.

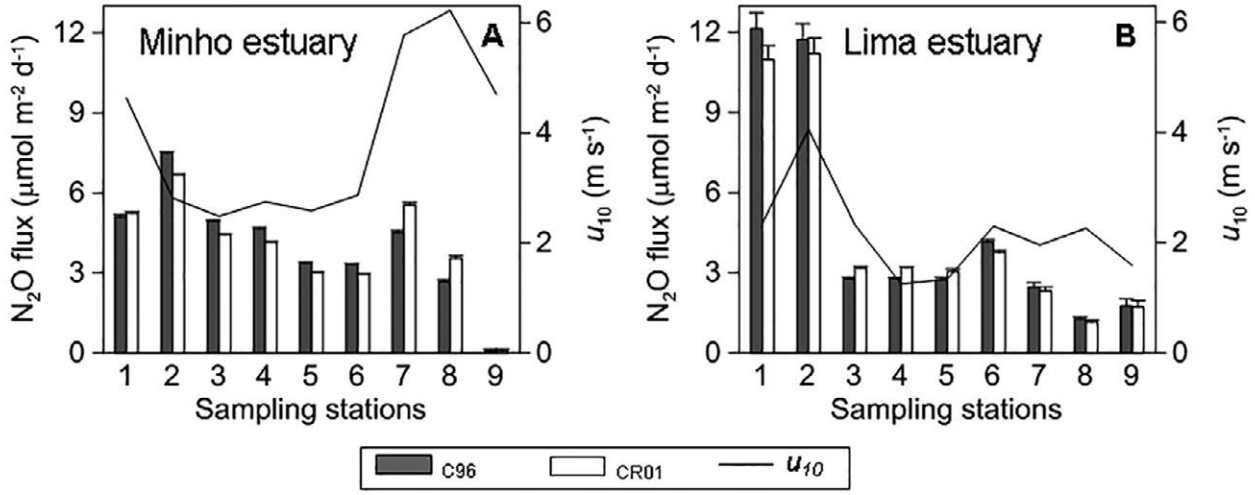

Fig. 6. – Water-air N_2O fluxes (bar charts) along the Minho (A) and Lima (B) estuaries. Wind speed (u_{10}) is represented by solid line (Error bars =±1 sd).

higher fluxes in the Lima estuary were mainly associated with the higher levels of N_2O observed in the upper estuary area (St.1 to St.3; Fig. 2), indicating that the low salinity zone (0-5) is an important source of N_2O to the atmosphere.

Using the two different parameterizations (C96, RC01) to calculate the gas transfer coefficients, the averaged N_2O water-air fluxes from Minho estuary to the atmosphere ranged between 4.0±3.3 µmol m^{-2} d^{-1} (RC01) and 4.1±2.8 µmol m^{-2} d^{-1} (C96), corresponding to a mean N_2O concentration of 11.3±1.3 nmol L^{-1} (132±22% saturation) and a mean wind speed of 3.9±0.1 m s^{-1}. Slightly higher N_2O water-air fluxes were found in the Lima estuary, with averaged values ranging between 4.7±1.9 µmol m^{-2} d^{-1} (RC01) and 5.0±2.0 µmol m^{-2} d^{-1} (C96), corresponding to a higher mean N_2O concentration of 13.7±1.6 nmol L^{-1} (153±26% saturation) and a lower mean wind speed level (2.4±0.1 m s^{-1}).

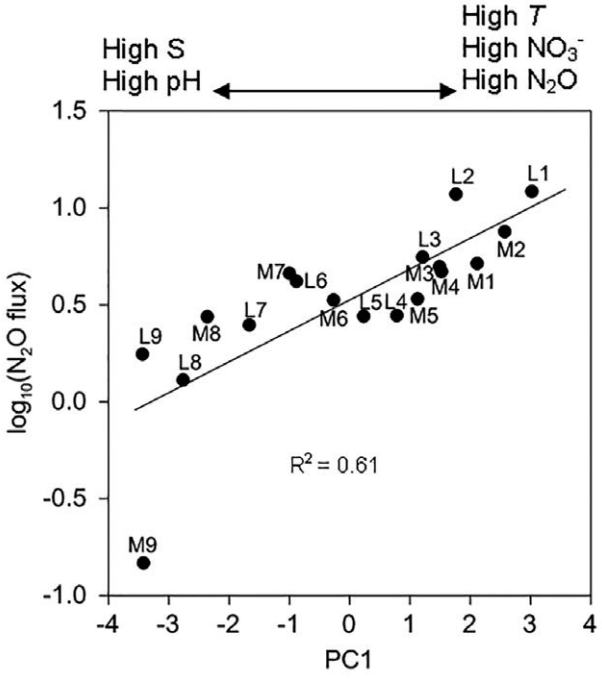

Fig. 7. – Relationship between log-transformed values of N_2O fluxes and the first principal component in the Minho and Lima estuaries. R^2, correlation coefficient. Variable trends are indicated along the top of the figure.

N_2O fluxes from the Minho and Lima estuaries were regressed versus the first two PCs' ordination of station scores to test their ability to predict the fluxes. We found out that only PC1 showed to be correlated with N_2O flux, with a strong positive correlation (R^2=0.61) (Fig. 7). N_2O flux also increased along a gradient of increasing NO_3^-, T and N_2O. These results suggest that future global changes in these parameters will result in an increase of N_2O flux in these estuarine systems.

DISCUSSION

The present study reveals that the Minho and Lima estuaries, particularly the upper reaches, behave differently regarding N_2O levels, sources and fluxes. N_2O distribution exhibits a pronounced spatial variability in both estuaries but in the Lima estuary concentrations are higher than in Minho. The Lima River was the main N_2O contributor to the Lima estuary, whereas the occurrence of nitrification seems to represent an additional N_2O source within the Minho estuary. As NH_4^+ is a primary substrate for nitrification, low NH_4^+ concentration may limit nitrification. It has been suggested that the AOA and AOB niches are defined by ammonium concentrations (Martens-Habbena et al. 2009), with AOA dominating in ammonia-limited acid, whereas AOB have a tolerance of high ammonia concentrations.

Though no information on benthic AOA and AOB communities along the Minho and Lima estuaries is available, NH_4^+ concentration in the upper Minho estuary (maximum 4.4 µmol L^{-1}) seems more suitable for the occurrence of nitrification than in the Lima estuary (maximum 1.8 µmol L^{-1}). Nitrification reactions typically happen within a DO range of 15.6-78.0 µmol L^{-1}, and the Minho and Lima surface waters were well

Table 4. – N_2O saturation, water-air fluxes and emissions for several world estuaries. Estimated using the relationships of [(a)] Clark et al. (1995); [(b)] Carini et al. (1996); [(c)] Raymond and Cole (2001); [(e)] unpublished data.

Estuaries	Area (km^2)	Nitrous Oxide (N_2O) Saturation (%)	Water-air fluxes ($\mu mol\ m^{-2}\ d^{-1}$)	Emission (Mg N_2O-N yr^{-1})	Sampling survey	Reference
Loire, France	41	84 - 271	14.3 [(a)]	6.1	Single	de Wilde and de Bie (2000)
Scheldt, Belgium	269	710	66.6 [(a)]	1.8×10^2	Seasonal	de Wilde and de Bie (2000)
Tagus, Portugal	320	101 - 147	–1.0 - 10.4 [(a)]	12.8 - 16.0 [(e)]	Seasonal	Gonçalves et al. (2010)
Humber, UK	303.6	100 - 4250	76.6 [(a)]	2.5×10^2	Seasonal	Barnes and Upstill-Goddard (2011)
Tay, UK	121.3	100 - 118	2.5 [(a)]	2.5	Single	Barnes and Upstill-Goddard (2011)
Tyne, UK	7.9	98 - 280	7.5	0.37	Seasonal	Barnes and Upstill-Goddard (2011)
Ems, Germany	162	181 - 1794	76.7 [(a)]	1.3×10^2	Single	Barnes and Upstill-Goddard (2011)
Gironde, France	442	120 - 463	25.5 [(a)]	1.2×10^2	Seasonal	Barnes and Upstill-Goddard (2011)
Douro, Portugal	2.4	280 - 650	74.7 [(a)]	1.9	Single	Barnes and Upstill-Goddard (2011)
Sado, Portugal	180	91 - 162	–2.2 [(b)]- 3.8 [(c)]	3.7 - 4.4	Single	Gonçalves et al. (2015)
Guadalete, Spain	=	96 - 2174	–0.1 - 313.2	-	Seasonal	Burgos et al. (2015)
Pearl River, China	2789	101 - 3800	0.1 - 733	1.35×10^3	Seasonal	Lin et al. (2016)
Minho, Portugal	23	101 - 166	4.0 [(c)] - 4.1 [(b)]	0.94 - 0.96	Single	This study
Lima, Portugal	5.4	113 - 227	4.7 [(c)] - 5.0 [(b)]	0.26 - 0.28	Single	This study

above these concentrations, leading to nitrification conditions. However, only in the upper Minho do correlations found between ΔN_2O and AOU, NH_4^+ and NO_2^- suggest that nitrification may have been acting as an NH_4^+ sink and a source of N_2O. The calculated biological N_2O yield (0.060 nmol per µmol O_2 consumed) falls within the range observed in marine systems and in particular in the Atlantic off the Iberian coast (Nevison et al. 2003). The effect of salinity on nitrification is well documented and in many estuarine systems nitrification rates are highest at lower and intermediate salinities (Bianchi et al. 1999, Teixeira et al. 2013). Our results from the upper Minho are in accordance with these findings, as the potential nitrification occurred at low salinity (between ~2 and ~10). The community composition of nitrifying microbes is very dependent on salinity, but a combination of other environmental factors may shape AOB diversity along an estuary (Mosier et al. 2008).

pH may regulate nitrification, and Wild et al. (1971) found an ideal pH range for nitrification between 7.5 and 8.5. As nitrifiers are known to decrease pH, the sharp negative correlation found between pH and ΔN_2O in the pH range 7.2-7.4 in the upper Minho estuary (Fig. 3E) may be a direct result of nitrification. N_2O saturation values ranging from 101% to 166% in the Minho estuary and 113% to 227% in the Lima estuary indicate that both estuaries behave as a potential N_2O source to the atmosphere. Positive N_2O water-air fluxes prevailed in all sampling stations, decreasing in general from upper to lower estuaries. However, this tendency was more pronounced in the Lima estuary, where higher N_2O fluxes in the river-influenced area were about twice (~12.0 µmol m^{-2} d^{-1}; C96) those observed in the upper part of the Minho estuary (~6.0 µmol m^{-2} d^{-1}; C96). It is likely that a greater turbulence of Minho upper estuary waters leads to a more rapid degassing of N_2O to the atmosphere in this part of the system.

Estimated N_2O fluxes were similar to those reported from the Portuguese Tagus and Sado estuaries (Gonçalves et al. 2010, 2015) (Table 4) but much lower than that of the Douro estuary (Barnes and Upstill-Goddard 2011). These fluxes were also much lower than those reported from the Scheldt estuary in Belgium, the Ems estuary in Germany, the Humber estuary in the UK, the Guadalete estuary in Spain and the Pearl River estuary in China (Table 4).

Though N_2O water-air fluxes were obtained during a single sampling, aware of the seasonal variability that characterizes these estuarine systems, we estimated the annual contribution of Minho and Lima estuaries to the global N_2O emissions. Taking into account estuarine areas (23 km^2 for Minho and 5.4 km^2 for Lima) and the estimated mean N_2O fluxes, we extrapolated an emission of 0.94-0.96 Mg N_2O-N yr^{-1} (estimated using RC01 and C96, respectively) for the Minho estuary and 0.26-0.28 Mg N_2O-N yr^{-1} (RC01 and C96, respectively) for the Lima estuary.

On a global perspective, estimated N_2O emissions from the Minho and Lima estuaries (<1.0 Mg N_2O-N yr^{-1}) represent a reduced fraction (<0.02%) of emissions from European estuaries (6.8 Gg N_2O yr^{-1}, Barnes and Upstill-Goddard 2011). Nevertheless, being aware of our unique seasonal sampling, and particularly the higher values of N_2O emissions measured in winter spring and in other Portuguese estuaries (Gonçalves et al. 2010), more studies assessing the seasonal variability of N_2O emissions in our systems are needed.

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